

What is claimed:

1. An embryoid body cell population, wherein said embryoid body cell population can be derived by culturing an embryonic stem cell population in an embryoid body cell medium comprising platelet-poor fetal bovine serum.
2. The embryoid body cell population of Claim 1, wherein said embryoid body cell medium comprises about 15% platelet-poor fetal bovine serum.
3. The embryoid body cell population of Claim 1, wherein said embryoid body cell medium does not include leukocyte inhibitory factor.
4. The embryoid body cell population of Claim 1, wherein said step of culturing is performed from about 1 day to about 7 days.
5. The embryoid body cell population of Claim 1, wherein said step of culturing is from about 3 days to about 4 days.
- 5 6. The embryoid body cell population of Claim 1, wherein said embryoid body cell population comprises cells of a cellular lineage selected from the group consisting of mesodermal cellular lineage, ectodermal cellular lineage and endodermal cellular lineage.
7. The embryoid body cell population of Claim 1, wherein said embryoid body cell population comprises cells of a cellular lineage selected from the group consisting of

hematopoietic lineage, endothelial lineage, muscle cell  
5 lineage, epithelial cell lineage and neural cell lineage.

8. The embryoid body cell population of Claim 1, wherein said embryoid body cell population comprises cells of a cellular lineage selected from the group consisting of erythroid lineage, endothelial lineage, and leukocyte lineage.

9. The embryoid body cell population of Claim 1, wherein said embryoid body cell population comprises cells of a cellular lineage selected from the group consisting of erythroid lineage, macrophage lineage, neutrophil lineage,  
5 mast cell lineage, megakaryocyte lineage, natural killer cell lineage, eosinophil lineage, T cell lineage, endothelial cell lineage and B cell lineage.

10. The embryoid body cell population of Claim 1, wherein said embryoid body cell population comprises a cell having a morphology substantially similar to one or more cells depicted in Fig.2, cell colony A.

11. The embryoid body cell population of Claim 1, wherein said embryoid body cell population can be cultured in an effective medium comprising an endothelial growth factor and an erythroid growth factor to obtain a mixed population of  
5 endothelial cells and erythroid cells.

12. The embryoid body cell population of Claim 1, wherein said embryoid body cell population can be cultured under effective conditions including an effective medium

comprising platelet-poor fetal bovine serum, methyl cellulose,  
5 and a growth factor selected from the group consisting of C-  
kit ligand, interleukin 1, interleukin 3, interleukin 6,  
interleukin 11, erythropoietin, vascular endothelial growth  
factor, and mixtures thereof, to obtain a cell population  
comprising embryonic blast cells.

13. A method to produce a cell type selected from the group consisting of mesodermal cells, ectodermal cells, endodermal cells, and progenitors and progeny thereof, comprising:

- 5           a) selecting a desired cell type; and  
          b) culturing an embryoid body cell population under conditions suitable to obtain said cell type.

14. The method of Claim 13, wherein said embryoid body cell population is derived by culturing a population of embryonic stem cells from about 1 day to about 7 days.

15. The method of Claim 13, wherein said embryoid body cell population is derived by culturing a population of embryonic stem cells from about 3 days to about 4 days in an embryoid body cell medium.

16. The method of Claim 15, wherein said embryonic stem cell population is derived from a mammalian embryo.

17. The method of Claim 13, wherein said embryoid body cell population is derived by culturing a population of embryonic stem cells in the presence of platelet-poor fetal bovine serum.

18. The method of Claim 13, wherein said embryoid body cell population is derived by culturing a population of embryonic stem cells in the presence of about 15% platelet-poor fetal bovine serum.

19. The method of Claim 13, wherein said embryoid body cell population is derived by culturing a population of embryonic stem cells in the absence of leukocyte inhibitory factor.

20. The method of Claim 13, wherein said cell type is selected from the group consisting of cells of hematopoietic lineage, cells of endothelial lineage, cells of muscle lineage, cells of epithelial lineage and cells of neural  
5 lineage.

21. The method of Claim 13, wherein said step of culturing comprises culturing said embryoid body cell population in a medium comprising erythropoietin and vascular endothelial growth factor to obtain a cell population  
5 comprising endothelial cells and erythroid cells.

22. The method of Claim 13, wherein said step of culturing comprises culturing said embryoid body cell population in a medium comprising a growth factor selected from the group consisting of C-kit ligand, interleukin 1,  
5 interleukin 3, interleukin 6, interleukin 11, erythropoietin, vascular endothelial growth factor, homologues thereof, and mixtures thereof, to obtain a cell population comprising embryonic blast cells.

23. The method of Claim 22, wherein said method further comprises culturing said embryonic blast cell population in a

medium comprising interleukin 7, insulin-like growth factor 1 and C-kit ligand to obtain a BLAST-LYM cell population.

24. The method of Claim 23, wherein said method further comprises culturing said BLAST-LYM cell population in a fetal thymi culture to obtain a T cell population.

25. The method of Claim 23, wherein said method further comprises culturing said BLAST-LYM cell population with bone marrow stromal cells to obtain a B cell population.

26. The method of Claim 22, wherein said method further comprises culturing said embryonic blast cell population in a medium comprising a growth factor selected from the group consisting of C-kit ligand, interleukin 1, interleukin 3, 5 interleukin 6, interleukin 7, interleukin 11, erythropoietin, vascular endothelial growth factor, GM-CSF, G-CSF, M-CSF, homologues thereof, and mixtures thereof, to obtain a BLAST-NEM cell population.

27. An embryonic blast cell population, wherein said  
 blast cell population can be derived by culturing an embryoid  
 body cell population under conditions effective to produce  
 said embryonic blast cell population, said conditions  
 5 comprising an embryonic blast cell medium.

*E* 28. The ~~embryonic~~ <sup>pluripotent</sup> blast cell population of Claim 27,  
 wherein said embryonic blast cell medium comprises at least  
 one growth factor selected from the group consisting of a  
 hematopoietic cell growth factor and an endothelial cell  
 5 growth factor.

*E* 328. The ~~embryonic~~ <sup>pluripotent</sup> blast cell population of Claim 27,  
 wherein said embryonic blast cell medium comprises at least  
 one growth factor selected from the group consisting of C-kit  
 ligand, interleukin 1, interleukin 3, interleukin 6,  
 5 interleukin 11, erythropoietin, vascular endothelial growth  
 factor, and homologues thereof.

*E* 430. The ~~embryonic~~ <sup>pluripotent</sup> blast cell population of Claim 27,  
 wherein said embryonic blast cell medium comprises a mixture  
 of C-kit ligand, interleukin 1, interleukin 6 and interleukin  
 11, or a mixture of C-kit ligand, interleukin 1,  
 5 erythropoietin and vascular endothelial growth factor.

*E* 531. The ~~embryonic~~ <sup>pluripotent</sup> blast cell population of Claim 27,  
 wherein said embryonic blast cell medium comprises C-kit  
 ligand.

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32. The ~~embryonic~~ <sup>pluripotent</sup> blast cell population of Claim 27,  
wherein said embryonic blast cell medium comprises platelet-  
poor fetal bovine serum.

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33. The ~~embryonic~~ <sup>pluripotent</sup> blast cell population of Claim 27,  
wherein said embryonic blast cell medium comprises platelet-  
poor fetal bovine serum and a growth factor selected from the  
group consisting of endothelial growth factors, hematopoietic  
5 growth factors, and mixtures thereof.

Sub.E 34. ~~The embryonic blast cell population of Claim 27,~~  
wherein said embryonic blast cell population can be derived by  
culturing said embryoid body cell population from about 2 days  
to about 15 days.

35. ~~The embryonic blast cell population of Claim 27,~~  
wherein said embryonic blast cell population can be derived by  
culturing said embryoid body cell population from about 3 days  
to about 6 days.

36. The embryonic blast cell population of Claim 27,  
wherein said embryonic blast cell population comprises a cell  
capable of developing into a cell type selected from the group  
consisting of primitive erythroid cells, definitive erythroid  
5 cells, macrophages, neutrophils, mast cells, T cells,  
endothelial cell, B cells, natural killer cells,  
megakaryocytes, eosinophils and progenitors and progeny  
thereof.



Sub E3  
37. The embryonic blast cell population of Claim 27, wherein said embryonic blast cell population can be cultured in a medium comprising one or more growth factors selected from the group consisting of C-kit ligand, interleukin 1, 5 interleukin 3, interleukin 6, interleukin 11, erythropoietin, vascular endothelial growth factor, GM-CSF, G-CSF, and M-CSF, to obtain a BLAST-NEM cell population.

38. The embryonic blast cell population of Claim 27, wherein said embryonic blast cell population can be cultured in a medium comprising one or more growth factors selected from the group consisting of interleukin 7, insulin-like 5 growth factor 1, and C-kit ligand to obtain a BLAST-LYM cell population.

39. The embryonic blast cell population of Claim 27, wherein said embryonic blast cell population can be cultured in medium comprising interleukin 7, insulin-like growth factor 1, and C-kit ligand, to obtain a BLAST-LYM cell population.

E 13/40. The ~~embryonic~~ pluripotent blast cell population of Claim 12/39, wherein said BLAST-LYM cell population can be cultured in a fetal thymi culture to obtain a T cell population.

14/41. The ~~embryonic~~ pluripotent blast cell population of Claim 12/39, wherein said BLAST-LYM cell population can be cultured with bone marrow stromal cells to obtain a B cell population.

E 15/42. The ~~embryonic~~ pluripotent blast cell population of Claim 1/21, wherein said population comprises a cell having a morphology

substantially similar to one or more cells depicted in Fig. 2,  
cell colony B.

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*Sub.E4*  
~~43.~~ A method for obtaining a population comprising embryonic blast cells, said method comprising culturing a population of embryoid body cells under conditions effective to obtain embryonic blast cells, said conditions comprising an embryonic blast cell medium.

~~18~~ 44. The method of Claim ~~43~~<sup>17</sup>, wherein said embryonic blast cell medium comprises a growth factor selected from the group consisting of a hematopoietic cell growth factor, an endothelial cell growth factor and a mixture thereof.

~~19~~ 45. The method of Claim ~~43~~<sup>17</sup>, wherein said embryonic blast cell medium comprises one or more growth factors selected from the group consisting of C-kit ligand, interleukin 1, interleukin 3, interleukin 6, interleukin 11, erythropoietin, vascular endothelial growth factor, and homologues thereof.

~~20~~ 46. The method of Claim ~~43~~<sup>17</sup>, wherein said embryonic blast cell medium comprises a mixture of C-kit ligand, interleukin 1, interleukin 6 and interleukin 11, or a mixture of C-kit ligand, interleukin 1, erythropoietin and vascular endothelial growth factor.

~~21~~ 47. The method of Claim ~~43~~<sup>17</sup>, wherein said embryonic blast cell medium comprises platelet-poor fetal bovine serum.

~~22~~ 48. The method of Claim ~~43~~<sup>17</sup>, wherein said embryonic blast cell medium comprises about 10% platelet-poor fetal bovine serum.

23<sup>17</sup>~~48~~. The method of Claim ~~43~~<sup>17</sup>, wherein said embryonic blast cell medium comprises platelet-poor fetal bovine serum, methyl cellulose, and a growth factor selected from the group consisting of endothelial growth factors, hematopoietic growth factors, and mixtures thereof.

24<sup>17</sup>~~50~~. The method of Claim ~~43~~<sup>17</sup>, wherein said step of culturing is performed at a cell density of from about  $1 \times 10^5$  cells to about  $7.5 \times 10^5$  cells per milliliter of culture medium.

25<sup>17</sup>~~51~~. The method of Claim ~~43~~<sup>17</sup>, wherein said step of culturing is performed is cultured at a cell density of from about  $5 \times 10^5$  cells to about  $6 \times 10^5$  cells per milliliter of culture medium.

26<sup>17</sup>~~52~~. The method of Claim ~~43~~<sup>17</sup>, wherein said step of culturing is performed is cultured at a cell density of from about  $2.5 \times 10^5$  cells to about  $5 \times 10^5$  cells per milliliter of culture medium.

E 27<sup>17</sup>~~53~~. The method of Claim ~~43~~<sup>17</sup>, wherein said step of culturing ~~can be~~<sup>is</sup> performed from about 2 days to about 15 days.

E 28<sup>17</sup>~~54~~. The method of Claim ~~43~~<sup>17</sup>, wherein said step of culturing ~~can be~~<sup>is</sup> performed from about 3 days to about 10 days.

E 29<sup>17</sup>~~55~~. The method of Claim ~~43~~<sup>17</sup>, wherein said step of culturing ~~can be~~<sup>is</sup> performed from about 3 days to about 6 days.

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E 56. The method of Claim 43, wherein said embryoid body  
E cell population ~~can be~~<sup>is</sup> derived by culturing a population of  
said embryonic stem cells from about 1 day to about 7 days.

57. The method of Claim 43, wherein said embryonic blast  
cell population comprises cells capable of developing into a  
cell type selected from the group consisting of primitive  
erythroid cells, definitive erythroid cells, macrophages,  
5 neutrophils, mast cells, T cells, endothelial cell, B cells,  
natural killer cells, megakaryocytes, eosinophils and  
progenitors and progeny thereof.

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E 58. The method of Claim 43, wherein said step of  
culturing ~~can be~~<sup>is</sup> performed at about 37°C in an about 5% CO<sub>2</sub>  
environment.

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Sub. ES 59. The method of Claim 43, wherein said method  
comprises:

a) culturing an embryonic stem cell population in  
an embryoid body cell medium from about 3 days to about 4 days  
5 to obtain embryoid body cell population; and

b) culturing said embryoid body cell population in  
an embryonic blast cell medium from about 3 days to about 6  
days to obtain a cellular population comprising embryonic  
blast cells.

60. A mixed population of endothelial cells and erythroid cells, wherein said mixed population can be derived by culturing an embryoid body cell population in an effective medium comprising an endothelial cell growth factor and an erythroid cell growth factor.

61. The mixed population of Claim 60, wherein said growth factor is selected from the group consisting of C-kit ligand, erythropoietin, vascular endothelial growth factor, a growth factor produced by said embryoid body cell, homologues thereof, and mixtures thereof.

62. The mixed population of Claim 60, wherein said medium comprises erythropoietin and vascular endothelial growth factor.

63. The mixed population of Claim 60, wherein said mixed population is derived by culturing a population of embryoid body cells from about 1 day to about 7 days.

64. The mixed population of Claim 60, wherein said embryoid body cell population is derived by culturing a population of embryonic stem cells from about 3 days to about 4 days in an embryoid body cell medium.

65. The mixed population of Claim 60, wherein said embryoid body cell population is cultured at a cell density of from about  $5 \times 10^4$  cells to about  $7.5 \times 10^5$  cells per milliliter of culture medium.

66. The mixed population of Claim 60, wherein said population comprises cells having morphologies substantially similar to one or more cells depicted in Fig. 4, cell B and cell C.

67. The mixed population of Claim 60, wherein said mixed population comprises a cell that can be stained with von Willebrand factor and that can take up diI-acetylated-low density lipoproteins.

68. A method to produce a mixed population of endothelial and erythroid cells, comprising culturing an embryoid body cell population in an effective medium comprising an endothelial cell growth factor and an erythroid  
5 cell growth factor.

69. The method of Claim 68, wherein said medium comprises erythropoietin and vascular endothelial growth factor.

70. The method of Claim 68, wherein said step of culturing is performed at a cell density of from about  $5 \times 10^4$  cells to about  $7.5 \times 10^5$  cells per milliliter of culture medium.

71. The method of Claim 68, wherein said step of culturing is performed at a cell density of from about  $2 \times 10^5$  cells to about  $5 \times 10^5$  cells per milliliter of culture medium.

72. The method of Claim 68, wherein said step of culturing is performed from about 6 days to about 11 days.



73. A BLAST-LYM cell population, wherein said population can be derived by culturing an embryonic blast cell population in a BLAST-LYM cell medium comprising one or more lymphoid cell growth factors.

74. The BLAST-LYM cell population of Claim 73, wherein said lymphoid cell growth factor is selected from the group consisting of interleukin 7, C-kit ligand, insulin-like growth factor 1, vascular endothelial growth factor, erythropoietin, a growth factor produced by said embryoid body cell, homologues thereof, and mixtures thereof.

75. The BLAST-LYM cell population of Claim 73; wherein said BLAST-LYM cell medium comprises interleukin 7, insulin-like growth factor 1 and C-kit ligand.

76. The BLAST-LYM cell population of Claim 73, wherein said embryonic blast cell population is derived by culturing a population of embryoid body cells from about 3 days to about 10 days.

77. The BLAST-LYM cell population of Claim 73, wherein said BLAST-LYM cell population can be cultured in a fetal thymi culture from about 2 weeks to about 3 weeks to obtain a T cell population.

78. The BLAST-LYM cell population of Claim 77, wherein said T cell population comprises T cells expressing a T cell receptor selected from the group consisting of  $\alpha\beta$  T cell receptor and  $\gamma\delta$  T cell receptor.

79. The BLAST-LYM cell population of Claim 73, wherein said BLAST-LYM cell population can be cultured with bone marrow stromal cells to produce lymphoid cells having rearranged immunoglobulin genes.

80. A method for obtaining a BLAST-LYM cell population comprising culturing an embryonic blast cell population with a BLAST-LYM cell medium comprising one or more lymphoid growth factor.

81. The method of Claim 80, wherein said lymphoid cell growth factor is selected from the group consisting of interleukin 7, C-kit ligand, insulin-like growth factor 1, vascular endothelial growth factor, erythropoietin, a growth  
5 factor produced by said embryoid body cell, homologues thereof and mixtures thereof.

82. The method of Claim 80, wherein said BLAST-LYM cell medium comprises interleukin 7, insulin-like growth factor 1 and C-kit ligand.

83. The method of Claim 80, wherein said step of culturing is performed for about 6 days.

84. A lymphoid cell population, wherein said lymphoid cell population can be derived by a method comprising:

a) culturing an embryonic blast cell population in a BLAST-LYM cell medium comprising one or more lymphoid cell growth factors to produce a BLAST-LYM cell population; and

b) culturing said BLAST-LYM cell population with cells selected from the group consisting of fetal thymic culture cells and bone marrow stromal cells to obtain said lymphoid cell population.

85. The lymphoid cell population of Claim 84, wherein said lymphoid cell population comprises cells selected from the group consisting of T cells and B cells.

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86. A BLAST-NEM cell population, wherein said BLAST-NEM cell population can be derived by culturing an embryonic blast cell population in an BLAST-NEM cell medium comprising a BLAST-NEM cell growth factor.

87. The BLAST-NEM cell population of Claim 86, wherein said growth factor is selected from the group consisting of C-kit ligand, interleukin 1, interleukin 3, interleukin 6, interleukin 11, erythropoietin, vascular endothelial growth factor, homologues thereof and mixtures thereof.

88. The BLAST-NEM cell population of Claim 86, wherein said BLAST-NEM cell medium comprises C-kit ligand, interleukin 1, interleukin 3, interleukin 6, interleukin 11, erythropoietin and vascular endothelial growth factor.

89. The BLAST-NEM cell population of Claim 86, wherein said BLAST-NEM cell population can be derived by culturing a population of embryonic blast cells from about 2 to about 12 days.

90. The BLAST-NEM cell population of Claim 86, wherein said BLAST-NEM cell population is capable of developing into a cell type selected from the group consisting of primitive erythroid cells, definitive erythroid cells, macrophages, mast cells, neutrophils, eosinophils, megakaryocytes, and progenitors and progeny thereof.

91. A method for obtaining a BLAST-NEM cell population comprising culturing an embryonic blast cell population in a BLAST-NEM cell medium comprising a BLAST-NEM growth factor.

92. The method of Claim 91, wherein said BLAST-NEM cell growth factor is selected from the group consisting of C-kit ligand, interleukin 1, interleukin 3, interleukin 6, interleukin 11, erythropoietin, vascular endothelial growth  
5 factor, homologues thereof and mixtures thereof.

93. The method of Claim 91, wherein said BLAST-NEM cell medium comprises C-kit ligand, interleukin 1, interleukin 3, interleukin 6, interleukin 11, erythropoietin and vascular endothelial growth factor.

94. The method of Claim 91, wherein said step of culturing is performed for about 2 days to about 12 days.

95. The method of Claim 91, wherein said step of culturing is performed for about 4 days to about 8 days.

96. The method of Claim 91, wherein said BLAST-NEM cell population comprises a cell capable of developing into a cell type selected from the group consisting of primitive erythroid cells, adult erythroid cells, macrophages, mast cells,  
5 neutrophils, megakaryocytes, and progenitors and progeny thereof.

97. A method to identify a compound expressed during the development of a population of embryonic blast cells, comprising characterizing the cellular composition of at least one cell contained in a population of cells selected from the group consisting of an embryonic stem cell population, an embryoid body cell population, an embryonic blast cell population, and intermediate cell populations thereof, to identify a compound expressed during the development of a population of embryonic blast cells.

98. The method of Claim 97, wherein said cellular composition comprises molecules selected from the group consisting of nucleic acids, proteins, carbohydrates, lipids and mixtures thereof.

99. The method of Claim 97, wherein said compound comprises a cellular marker capable of defining one or more populations of embryonic cells.

100. The method of Claim 97, wherein said compound is capable of regulating a cell function selected from the group consisting of maintaining the survival of a cell, inducing the propagation of a cell, and stimulating the differentiation of a cell.

101. The method of Claim 97, wherein said compound is selected from the group consisting of a cell surface molecule, a secreted molecule, a cytoplasmic signal transduction

molecule, a nucleic acid binding protein, and mixtures  
5 thereof.

102. The method of Claim 97, wherein said compound is  
selected from the group consisting of a cell surface receptor,  
a membrane-bound ligand, an adhesion protein, a carbohydrate  
moiety, a cytoplasmic signal transduction protein, a growth  
5 factor, a transcription factor, and mixtures thereof.

103. The method of Claim 97, wherein said population of  
cells is selected from the group consisting of an embryoid  
body cell population derived by culturing an embryonic stem  
cell population from about 1 day to about 7 days, and an  
5 embryonic blast cell population derived by culturing an  
embryoid body cell population from about 1 day to about 7  
days.

104. The method of Claim 97, wherein said step of  
characterizing comprises screening said cellular composition  
using a method selected from the group consisting of direct  
nucleic acid hybridization, selective nucleic acid  
5 hybridization, nucleotide sequencing, antibody binding  
studies, cell culture survival assays, cell culture  
proliferation assays, kinase assays and protein:protein  
interaction assays.

105. The method of Claim 97, wherein said step of  
characterizing comprises screening said cellular composition  
for the presence of a nucleic acid encoding a protein selected



from the group consisting of an endothelial cell marker  
5 protein, a lymphoid cell marker protein, an epithelial marker  
protein and a hematopoietic precursor cell marker.

106. The method of Claim 97, wherein said step of  
characterizing comprises screening said cellular composition  
for the presence of a nucleic acid molecule encoding a protein  
selected from the group consisting of a stem cell leukemia  
5 protein, GATA-1, GATA-2, C-Myb, C-kit ligand, C-fms, Flk-1,  
beta-globin, betaH1-globin, brachyury, VLA-4 and LFA-1.

107. A formulation comprising culture medium isolated from a composition selected from the group consisting of a composition obtained by culturing a population of embryonic stem cells from about 1 day to about 7 days in an embryoid body cell medium to obtain an embryoid body cell population and a composition obtained by culturing a population of embryoid body cells for from about 3 to about 6 days in an embryonic blast medium to obtain embryonic blast cells.

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